Pacific Science (1994), vol. 48, no. 3: 263–272

Population Dynamics of Symbiotic Zooxanthellae in the Coral *Pocillopora damicornis* Exposed to Elevated Ammonium [$(NH_4)_2SO_4$] Concentrations[1]

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ABSTRACT: Division synchrony and growth rate of symbiotic zooxanthellae was investigated for populations living in colonies of the reef-building coral *Pocillopora damicornis* (Linnaeus) exposed to different concentrations of ammonium [$(NH_4)_2SO_4$] in seawater. Presence of low concentrations of ammonium (0.2 μM) did not affect (compared with corals growing in ammonium-stripped seawater) either division synchrony or growth rate. Exposure to higher concentrations of ammonium (20 or 50 μM), however, affected the population dynamics of the zooxanthellae residing in *P. damicornis*. Zooxanthellae in corals exposed to 20 μM ammonium had mitotic indices (percentage of total cells dividing) that were two to three times higher than mitotic indices of zooxanthellae in control (0.2 μM) corals. Although division of zooxanthellae was still phased in corals exposed to 20 μM ammonium, there were many more cells dividing out of phase compared with control corals. Division of zooxanthellae in corals exposed to 50 μM was not phased. Calculated growth rates of zooxanthellae exposed to 20 or 50 μM ammonium were higher than those representative of zooxanthellae living in control corals, although growth rate of both carbon and nitrogen pools was lower in 50 μM as compared with 20 μM ammonium. These data support the conclusion that the population dynamics of symbiotic zooxanthellae within *P. damicornis* are affected by concentrations of ammonium in seawater that are equal to or higher than 20 μM and that 50 μM ammonium concentrations may be toxic to some extent. These data taken in isolation, however, do not constitute an effective test of the hypothesis that zooxanthellae are limited by the supply of ammonium under ambient conditions and further emphasize the importance of enrichment studies concentrating on growth and nitrogen incorporation rates measured for the entire symbiotic association.

POPULATIONS OF SYMBIOTIC zooxanthellae are characterized by low growth rates relative to populations of cultured zooxanthellae (Wilkerson et al. 1983, Cook and D'Elia 1987). The low growth rates exhibited by symbiotic zooxanthellae have been cited as evidence of the host influence over the metabolism of symbiotic zooxanthellae, either passively (via restricted access to space and nutrients [Muscatine and Pool 1979, Cook and D'Elia 1987]) or actively (via host-specific mitogenic or cytogenic factors [Muscatine and Pool 1979]). A key experiment in identifying the importance of passive "control" mechanisms is to supply an excess of a particular nutrient and examine the response of the growth rate of the zooxanthellae. If an increase in the growth rate occurs after the addition of a nutrient (all else being equal), then the passive supply of the nutrient is a significant factor in explaining the low growth rate of zooxanthellae in hospice. The relative importance of the availability of a particular nutrient can then be determined by examining how closely the measured growth rate under surplus matches the maximum growth rate attained by symbiotic zooxanthellae under optimal growth conditions (e.g., in culture).

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Ammonium [$(NH_4)_2SO_4$] was supplied to the coral *Pocillopora damicornis* (Linnaeus) during the Hawaii Institute of Marine Biology (HIMB) experiment (see Stambler et al. [1994] and Muller-Parker et al. [1994]). Elevated ammonium has been shown to affect several characteristics of symbiotic zooxanthellae in reef-building corals, including their population density, photosynthetic rate, and chlorophyll *a* concentration (Høegh-Guldberg and Smith 1989, Muscatine et al. 1989, Stambler et al. 1991, Stimson and Kinzie 1991). It is interesting that the specific growth rate of zooxanthellae (μ_z) in these studies did not differ between treatments. Cook et al. (1988) demonstrated a stimulatory effect of adding NH_4Cl (together with phosphate) on the division rate of populations of symbiotic zooxanthellae in previously starved individuals of the sea anemone *Aiptasia pallida* (Verrill), but the effect of NH_4Cl on division was seen in only five of 23 anemones examined. The lack of a demonstrable effect of elevated nutrients on the division rates of zooxanthellae despite substantial increases in the population density of symbionts (seen at the end of the experiments reported by Cook et al. [1988]) suggests that the effect of elevated NH_4Cl on the division of zooxanthellae may be transitory and short-lived (Høegh-Guldberg and Smith 1989, Muscatine et al. 1989).

This paper reports the changes that occurred in the population dynamics of zooxanthellae in *P. damicornis* when exposed to elevated concentrations of $(NH_4)_2SO_4$ (as part of the U.S.–Israel 1991 HIMB workshop). To explore the possible transitory effect of increased ammonium availability, observations of the population dynamics of symbiotic zooxanthellae were made at both short (days) and long (weeks) time scales.

MATERIALS AND METHODS

Diel Patterns of Division by Symbiotic Zooxanthellae in Pocillopora damicornis

The diel patterns of cell division of symbiotic zooxanthellae in *P. damicornis* were investigated by sampling coral colonies incubated for 8 weeks in four different concentrations of $(NH_4)_2SO_4$ in seawater (three coral colonies per treatment [see Stambler et al. (1994) for details of maintenance of corals during the experiment]) every 3 hr for 48 hr. Small branch tips (≤ 1.5 cm) were broken off the coral colonies during each sampling time and were placed in 10% formalin in seawater. After fixing the tissues in this solution for at least 5 hr, the branch tips were decalcified in 4% nitric acid (in seawater) as described by Stimson (1990). The decalcified tissue was then rinsed in seawater and homogenized using a glass-glass homogenizer in a small volume of seawater (0.5 ml). The number of dividing cells in 1000 cells was counted using a hemacytometer (Bright-line, American Optical Corp.). Mitotic index was calculated by expressing the number of dividing cells as a percentage of the total number of cells inspected.

The mitotic index of symbiotic zooxanthellae in *P. damicornis* was also examined as a function of exposure time to elevated concentrations of ammonium. Three colonies of *P. damicornis* that had been exposed to ammonium for 0 (collected within 24 hr [=freshly collected]), 2, 4, 6, and 8 weeks were sampled at 0230 hr (time of peak division), and the mitotic index was measured, as described above. In a separate set of experiments, three colonies were moved between treatments to investigate the short-term influences of changes in concentrations of ammonium. Three different treatments were used as follows: corals were moved from the same treatment and back again, or they were moved from high to low concentrations or from low to high concentrations.

Calculation of Specific Growth Rates (μ_z), Duration of Division (t_d), and Changes in the Carbon and Nitrogen Pools of Symbiotic Zooxanthellae in Pocillopora damicornis

The specific growth rate of the zooxanthellae (μ_z) in *P. damicornis* was calculated using formulas for phased and unphased division of populations of zooxanthellae as described by Wilkerson et al. (1983). The duration of

division (t_d) was also calculated using the method outlined by Wilkerson et al. (1983) for those populations of zooxanthellae exhibiting phased cell division. Changes in the standing stock of carbon and nitrogen within the symbiotic zooxanthellae during nutrient enrichment were calculated by multiplying μ_z by the population density and the carbon (C) or nitrogen (N) content of the zooxanthellae (Muller-Parker et al. 1994).

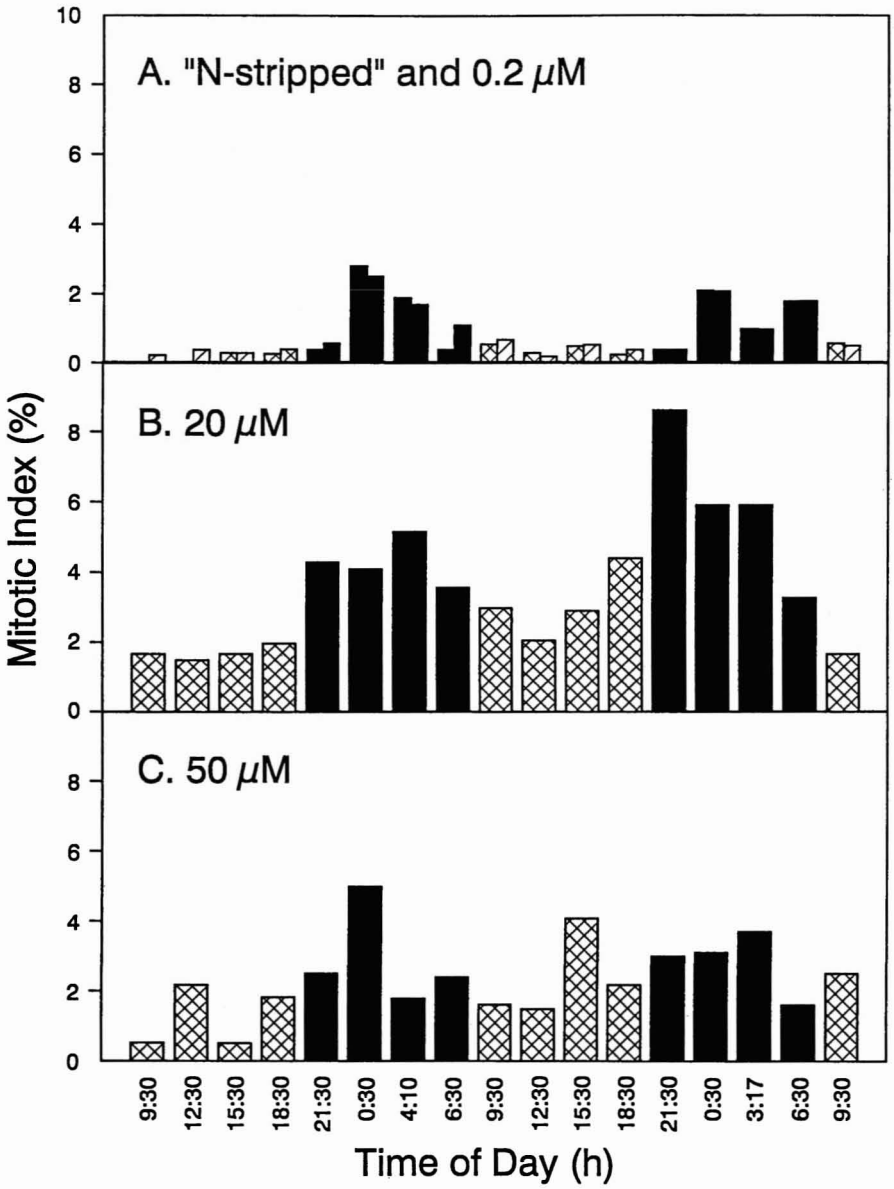

FIGURE 1. Mitotic index (percentage total zooxanthellae dividing) as a function of time of day for colonies of the coral *Pocillopora damicornis* exposed to: *A*, N-stripped (control) and ambient (0.2 μM); *B*, 20 μM; and *C*, 50 μM ammonium. Black bars indicate hours of darkness (night). No difference was detected between sampling times ($n = 3$). The pooled standard error of the mean averaged 8.1% of the mean.

RESULTS

Diel Pattern and the Duration of Division (t_d) of Symbiotic Zooxanthellae

Symbiotic zooxanthellae in *P. damicornis* given low levels of dissolved ammonium ("N-stripped" and 0.2 μM, ambient [measured concentrations given in Stambler et al. (1994)]) showed a diel pattern of division that was phased and had maxima that occurred between 0000 hr (midnight) and 0600 hr (Figure 1*A*). Maximum mitotic indices (f_{max}) were 2.81 and 2.50% on day 1 (first 24 hr of measurement) and were 2.10% on day 2 (second 24 hr) in both cases (Table 1). Mitotic indices at times outside the period of peak division activity were low (<0.5%). Division was phased in *P. damicornis* incubated in 20 μM ammonium, although the number of dividing cells found between peak periods (Figure 1*B*) was approximately five-fold greater than that seen among zooxanthellae in N-stripped or 0.2-μM treatments. Maximum division rates were also two to three times greater (Figure 1*A* and *B*, Table 1). When corals were treated with 50 μM ammonium, division rates were not phased, although the highest value for mitotic index was recorded at 0030 hr (Figure 1*C*), which is the beginning of the period of maximal division for zooxanthellae in *P. damicornis* when division was phased in other treatment conditions.

The mitotic index of symbiotic zooxanthellae in *P. damicornis* was also examined as a function of exposure to surplus ammonium (Figure 2). Zooxanthellae in *P. damicornis* receiving 0.2, 20, and 50 μM ammonium for 2 weeks had higher mitotic indices than those of freshly collected *P. damicornis*. Although the mitotic index of zooxanthellae in the 0.2-μM treatments did not vary with exposure ranging from 2 to 8 weeks, the mitotic index of zooxanthellae in *P. damicornis* receiving 20 and 50 μM ammonium increased as the length of their exposure to ammonium increased (Figure 2).

The duration of division (t_d) was calculated for those populations of zooxanthellae exhibiting phased cell division (Table 2). The time of division was comparable between treatments, although it was higher in treatments receiving 20-μM concentrations of ammonium (0.34, 0.39, and 0.66 day, for N-stripped, 0.2- and 20-μM treatments, respectively). The overall mean (for all treatments pooled on each day) was 0.47 and 0.46 days for the first and second day, respectively.

The Specific Growth Rate (μ_z) and the Growth Rate of Carbon and Nitrogen Pools in Symbiotic Zooxanthellae

The μ_z of zooxanthellae within the tissues of *P. damicornis* incubated in N-stripped or 0.2-μM ammonium seawater ranged between 0.021 and 0.028 day^{-1} (Table 1). Zooxanthellae within *P. damicornis* incubated in 20 and 50 μM ammonium had mitotic indices that ranged between 0.042 and 0.072 day^{-1}. When

TABLE 1

SPECIFIC GROWTH RATES OF ZOOXANTHELLAE (μ_z) IN *Pocillopora damicornis* 8 WEEKS AFTER THE BEGINNING OF NUTRIENT ENRICHMENT WITH AMMONIUM

AMMONIUM (μM)	MITOTIC INDEX (%)	μ_z (day^{-1})	C (μmol cm^{-2} day^{-1})	N (μmol cm^{-2} day^{-1})
0	2.81, 2.10	0.028, 0.021	1.32, 0.99	0.10, 0.08
0.2	2.50, 2.10	0.025, 0.021	0.89, 0.74	0.05, 0.04
20	4.29, 4.17	0.042, 0.057	3.09, 4.19	0.30, 0.40
50	2.09, 2.58	0.045, 0.055	1.55, 1.89	0.17, 0.21

NOTE: Rates were calculated using the equations described by Wilkerson et al. (1983) for phased (0, 0.2-, and 20-μM treatments; t_d = 0.46 days) and unphased cell division (50 μM). Values for mitotic index were taken from data set shown in Figure 1. The mean of two highest mitotic indices were averaged for each day for the treatments 0, 0.2, and 20 μM, whereas the overall mean mitotic index of each day was used for calculating μ_z in the 50-μM treatment. The first value of each pair represents data collected during the first 24 hr; the second value represents data collected in the second 24-hr period.

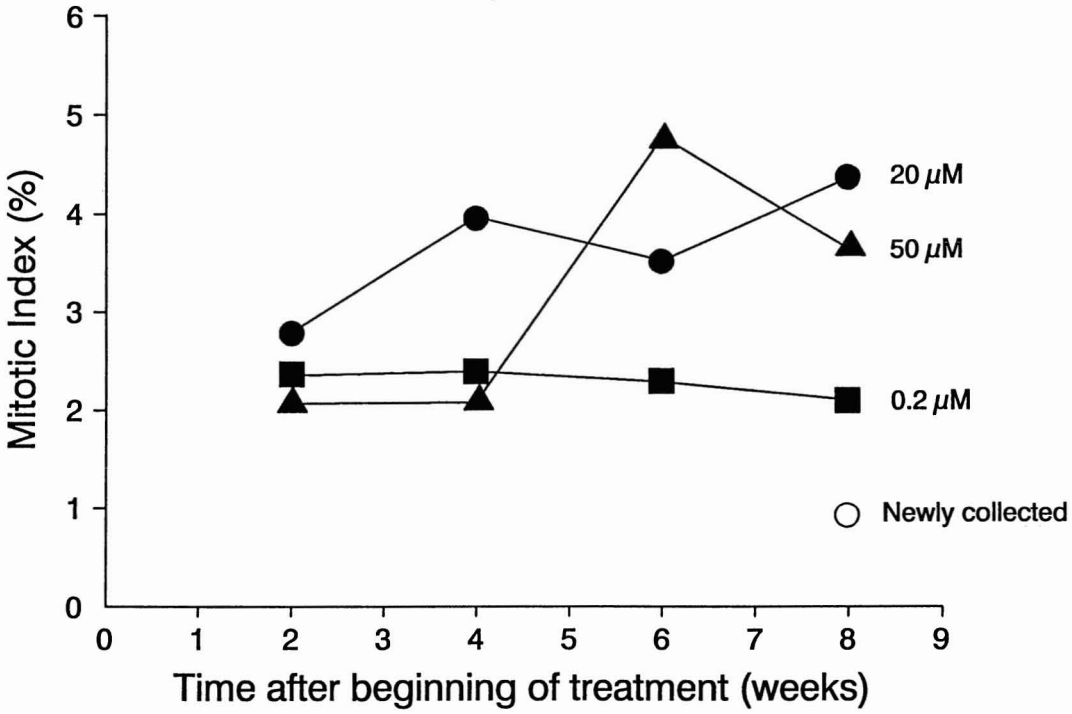

FIGURE 2. Mitotic index (percentage total zooxanthellae dividing) as a function of length of time that host corals (*Pocillopora damicornis*) were exposed to three different concentrations of ammonium in seawater. Concentrations are indicated at the end of each trajectory. Each point represents the mean of two measurements from different corals.

TABLE 2

DURATION OF DIVISION (t_d) OF SYMBIOTIC ZOOXANTHELLAE IN *Pocillopora damicornis* EXPOSED TO ELEVATED CONCENTRATIONS OF AMMONIUM

	CONCENTRATION OF AMMONIUM (μM)			
SPECIFIC GROWTH RATES (day^{-1})	0	0.2	20	50
Using equation 1:				
Day 1 (using f_{max})	0.028	0.025	0.042	N/A
Day 2 (using f_{max})	0.021	0.021	0.057	N/A
Using equation 2:	0.017	0.019	0.072	0.050
Duration of division (t_d):				
Day 1	0.29	0.35	0.78	N/A
Day 2	0.38	0.42	0.57	N/A

NOTE: Calculation is based on method outlined by Wilkerson et al. (1993, equations 1 and 2 indicated below). Refer to Table 1 for explanation of day 1 and day 2.

corals were transferred from one treatment to another, 24 hr before sampling, μ_z changed only when corals incubated in 20 μM were transferred to 0.2 μM (Table 3, simple t test, $P \leq 0.05$). These data indicate that the response time of μ_z to increased availability of ammonium is generally greater than 24 hr. The data also suggest that responses in μ_z

TABLE 3

SHORT-TERM EFFECT OF ELEVATED AMMONIUM

AMMONIUM (μM)	μ_z (day^{-1}) MEAN	SEM	95% confidence interval Min.	Max.
0.2 ⇒ 20	0.025	0.004	0.007	0.043
0.2 ⇒ 50	0.022	0.001	0.016	0.028
0.2 ⇒ 0.2	0.021	0.003	0.010	0.032
20 ⇒ 0.2	0.035*	0.003	0.023	0.046
20 ⇒ 20	0.061	0.002	0.054	0.069
50 ⇒ 0.2	0.023	0.001	0.020	0.026
50 ⇒ 50	0.030	0.004	0.013	0.046

NOTE: Specific growth rates (μ_z) calculated from mitotic indices of zooxanthellae populations in colonies of *Pocillopora damicornis* ($n = 3$) transferred to other treatment conditions. Samples were taken from the colonies at 0230 hr, 24 hr after transfer. Arrows indicate direction of transfer. Shown are means, standard errors of the mean (SEM), and 95% confidence intervals. Asterisk (*) indicates treatments where transfer resulted in a significant change ($P < 0.05$) relative to controls within each group.

may show hysteresis with respect to sudden increases versus decreases in the availability of ammonium.

The specific growth rate of zooxanthellae (μ_z) in *P. damicornis* was converted into a measure of the rate of growth of the organic carbon and nitrogen pools within the zooxanthellae, using other data collected during the study (Muller-Parker et al. 1994). Zooxanthellae isolated from corals incubated in relatively low concentrations of ammonium (≤ 0.2 μM) had organic carbon and nitrogen pools that grew at a rate of 0.99 μmol-C cm^{-2} day^{-1} (± 0.213 SD, $n = 4$, N-stripped and 0.2-μM treatments pooled) and 0.07 μmol-N cm^{-2} day^{-1} (± 0.024 SD, $n = 4$, Table 1), respectively, whereas zooxanthellae from corals incubated in 20 μM ammonium had organic carbon and nitrogen pools that grew at a rate of 3.64 μmol-C cm^{-2} day^{-1} (mean, $n = 2$) and 0.35 μmol-N cm^{-2} day^{-1} (mean, $n = 2$, Table 1), respectively. Growth of the carbon and nitrogen pools of zooxanthellae isolated from corals incubated in 50 μM ammonium were lower than those in corals incubated with 20 μM ammonium and were 1.72 μmol-C cm^{-2} day^{-1} (mean, $n = 2$) and 0.19 μmol-N cm^{-2} day^{-1} (mean, $n = 2$, Table 1), respectively.

DISCUSSION

Ammonium ions [$(NH_4)_2SO_4$], when supplied at concentrations of 20 μM and above, have a marked effect on the population dynamics of symbiotic zooxanthellae growing within the tissues of *P. damicornis*. The stimulatory effect of ammonium on the growth rate of zooxanthellae in *P. damicornis* is reflected ultimately in an increased density of zooxanthellae within the tissues of *P. damicornis* (Muller-Parker et al. 1994). One possible interpretation of these data is that the low growth rates of zooxanthellae in symbioses involving reef-building corals are a function of the restricted availability of ammonium (Høegh-Guldberg and Smith 1989, Muscatine et al. 1989). The validity of this idea is at the heart of the aims of the joint U.S.–Israel 1991 HIMB workshop.

Is Phased Cell Division and Low μ_z Characteristic of Populations of Symbiotic Zooxanthellae Found within Reef-building Corals?

The cell division of symbiotic zooxanthellae within the tissues of *P. damicornis* is phased, with a peak in the percentage of dividing cells (mitotic index) occurring between 0000 and 0600 hr. The cell division of zooxanthellae in the reef-building corals *Seriatopora hystrix* Dana (Høegh-Guldberg and Smith 1989), *Stylophora pistillata* Esper, *Fungia repanda*, and *P. damicornis* (Smith and Høegh-Guldberg 1987) from Lizard Island (northern sector of the Great Barrier Reef) was also phased. It is interesting that zooxanthellae in nine species of reef-building corals investigated by Wilkerson et al. (1988) in Discovery Bay, Jamaica, did not have phased cell division. Similar data were reported for *S. pistillata* from the Red Sea (Wilkerson et al. 1983). The exact reason for this difference between these studies is not clear. However, if elevated ammonium (50 μM) can lead to the

disappearance of phased division from populations of zooxanthellae in *P. damicornis* (as suggested here), then differences in how specimens are handled (e.g., aquarium ammonium concentration) may have important influences on the diel pattern of cell division measured for populations of symbiotic zooxanthellae. Until this peculiar difference is explored further, it is not possible to generalize that the division of symbiotic zooxanthellae is phased under normal conditions.

The specific growth rates (μ_z) of zooxanthellae found in reef-building corals are low relative to the μ_z exhibited by zooxanthellae under optimal growth conditions. Values of μ_z calculated from the mitotic indices measured in the study reported here ranged between 0.021 and 0.057 day^{-1} (calculated using equation 1 of Wilkerson et al. [1983]). Zooxanthellae from two other pocilloporid corals also had similar specific growth rates (*Seriatopora hystrix*: 0.040 to 0.082 day^{-1}; *Stylophora pistillata*: 0.028 to 0.032 day^{-1} [Høegh-Guldberg and Smith 1989]). These values are also similar to those reported for *S. pistillata* growing in the Red Sea (range: 0.013 to 0.094 day^{-1} [Wilkerson et al. 1983, Muscatine et al. 1985]). To date, the majority of specific growth rates determined for symbiotic zooxanthellae in reef-building corals fall into this range (Wilkerson et al. 1988). Given the potential for zooxanthellae to grow faster under more optimal conditions (e.g., at low population densities: up to 0.40 day^{-1} [Høegh-Guldberg and Hinde 1986]) or when cultured (up to 0.43 day^{-1} [Chang et al. 1983, Fitt and Trench 1983]), the μ_z characteristic of symbiotic zooxanthellae in reef-building corals is still relatively low under normal or low concentrations of ammonium.

Estimates of the rate of growth of organic carbon (C) and nitrogen (N) pools within the symbiotic populations of zooxanthellae were calculated using μ_z, the population density, and the amount of C and N per zooxanthella. Rates of growth of organic C (0.99 ± 0.213 μmol-C cm^{-2} day^{-1}) and N (0.07 ± 0.024 μmol-N cm^{-2} day^{-1}) were comparable with rates reported for zooxanthellae residing in the coral *Stylophora pistillata* (1.36 ± 0.686 μmol-C cm^{-2} day^{-1} and 0.17 ± 0.027 μmol-N cm^{-2} day^{-1} [data calculated using Tables 1 and 2 of Muscatine et al. (1989)]).

Are the Population Dynamics of Symbiotic Zooxanthellae in Pocillopora damicornis *Influenced by Elevated Concentrations of Ammonium* $[(NH_4)_2SO_4]$?

Ambient levels of ammonium (0.2 μM) had no detectable effect (relative to N-stripped seawater) on the growth dynamics of zooxanthellae growing within *P. damicornis*. At concentrations of 20 μM or greater, the zooxanthellae within *P. damicornis* showed increased maximum rates of division as well as a greater number of zooxanthellae dividing out of phase. When corals were exposed to 50 μM, the response of the zooxanthellae was complicated by a loss of division synchrony (phase) as well as a reduction (relative to the 20-μM treatment) in the number of zooxanthellae entering division at any one time. In both cases, however, the calculated specific growth rates were two to three times higher when compared with those calculated for the treatments receiving ammonium at concentrations of 0.2 μM or less. These data suggest that elevated ammonium can influence the population dynamics of symbiotic zooxanthellae in reef-building corals.

The relative importance of the availability of ammonium in determining the growth rate of zooxanthellae in *P. damicornis* can be assessed by comparing the maximal specific growth rates of zooxanthellae achieved in this study with the maximum growth rate attained by symbiotic zooxanthellae under more optimal growth conditions. Despite elevated concentrations of ammonium (20 μM) in the water surrounding the corals in this study, the maximum μ_z in this study was $<20\%$ of that when zooxanthellae are growing at very low population densities (0.40 day^{-1} [Høegh-Guldberg and Hinde 1986]) or when cultured (0.43 day^{-1} [Chang et al. 1983, Fitt and Trench 1983]). At a higher concentration (50 μM), the growth rate in this study was reduced relative to growth rates in 20 μM, and ammonium ions apparently have

a toxic effect. These data strongly suggest that ammonium is not the sole factor limiting the growth of zooxanthellae in hospice.

The direct influence of elevated ammonium on the specific growth rate of zooxanthellae (μ_z) within reef-building corals has not been reported before, although two studies (Høegh-Guldberg and Smith 1989, Muscatine et al. 1989) specifically measured μ_z of symbiotic zooxanthellae in the presence of elevated NH_4Cl. Those authors explained the unanticipated result of "no effect" as a function of the transient and short-lived effect of NH_4Cl on symbiotic zooxanthellae (as demonstrated by Fitt [1988] and Cook et al. [1988] for zooxanthellae in the hydrozoan *Myrionema amboinense* and the sea anemone *Aiptasia pallida* [Verrill]). This possibility does not appear to be supported here. The effect of ammonium on the mitotic index of zooxanthellae in *P. damicornis* was long-lived and was still present in populations that had been receiving ammonium for up to 8 weeks. A possible explanation of this discrepancy is that previous studies took single time-point samples based on the assumption that symbiotic zooxanthellae in low and high NH_4Cl treatments had similar diel patterns of cell division. Changes in the number of cells dividing out of phase (as seen in the study reported here), however, would lead to both under- and overestimated values of μ_z and a generally obscured picture of how μ_z responds to the increased availability of ammonium.

Stimulation of the specific growth rate of the zooxanthellae in corals exposed to 20 μM ammonium also translated into increased rates of growth of the N and C pools within the zooxanthellae. Specifically, rates of N and C pool growth were three to five times higher than those seen in corals exposed to ≤ 0.2 μM ammonium. Rates of N and C growth were lower (by a factor of 2) for zooxanthellae exposed to 50 μM ammonium (when compared with the 20-μM treatment), which corroborates other data (loss of division synchrony, and reduced N and C per cell in the 50-μM treatment [Muller-Parker et al. 1994]) that suggest that corals exposed to 50 μM are affected negatively by exposure to concentrations of ammonium in the vicinity of 50 μM.

Are Zooxanthellae in Reef-building Corals Limited by the Availability of Ammonium?

The general response of the population dynamics of zooxanthellae in *P. damicornis* reported here (increases in μ_z and the appearance of cells dividing out of phase) does not itself constitute proof that symbiotic zooxanthellae are limited by the supply of inorganic nitrogen. Most of the organic nitrogen synthesized by symbiotic zooxanthellae is probably translocated to the host (Muscatine and Cernichiari 1969, Trench 1979) and, therefore, by having been removed from the zooxanthella pools, is not actually accounted for by the observed changes in the growth rate of the zooxanthellae. Translocation also represents a confounding factor in the use of population dynamics to assess whether or not symbiotic populations are nitrogen-limited. For example, an equally tenable explanation for the observed increase in μ_z under elevated ammonium conditions is that translocation decreased in response to the toxic effects of ammonium on host metabolism, thereby leading to a greater retention of organic carbon for the growth of the zooxanthellae.

The resolution of the question as to whether symbiotic zooxanthellae are nitrogen-limited lies in the careful measurement of changes to the total size of the inorganic nitrogen pool of zooxanthellae in reef-building corals when exposed to elevated ammonium. The demonstration, for example, that ammonium uptake by reef-building corals is not saturated under ambient conditions would constitute confirmation of the proposal that the zooxanthellae are limited by the availability of ammonium under ambient conditions. This proposal is in fact supported by the observed increases in the total protein of corals in this study when exposed to elevated inorganic nitrogen (Muller-Parker et al. [1994]). This latter observation suggests that the growth of zooxanthellae is limited by the availability of inorganic nitrogen and that the observed increases in the frequency of dividing cells were due to bona fide increases in the

growth rate of zooxanthellae in response to the addition of inorganic nitrogen. This final point further highlights the importance of studies focusing on growth rates and nitrogen incorporation rates measured as a function of the entire symbiotic association for resolving the questions as to whether or not symbiotic zooxanthellae are limited by the availability of inorganic nitrogen under normal field conditions.

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